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Pretreatment of reed by wet-oxidation and subsequent utilization of the pretreated fibers for ethanol production

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INTRODUCTION

Common reed (*Phragmites australis*) is a large perennial grass native to wetland habitats at temperate and tropical latitudes. It usually forms vast belts along shores of freshwater lakes and rivers, but can also be found in marshes, swamps, or wet wastelands. Massive reed beds often have important ecological functions and are therefore to be protected, however, the rapid expansion of reed may just as well represent a serious threat to natural ecosystems.

Being one of the most widely distributed plant species on earth reed is used extensively in rural areas (most commonly as forage and bedding for livestock or as the structural material to build dwellings and fences or to wave ropes, coarse mats, and carrying nets. Due to its fast-growing properties and eventually high biomass yields reed can be recognized as a promising source of renewable energy crop. The few investigations carried out so far with common reed as an energy crop have been primarily focusing on the exploitation of its lignin content (25%, wt/wt) to produce solid fuel. Despite of its relatively high cellulose (33%, wt/wt) and hemicellulose (20%, wt/wt) contents no study has dealt yet with its potential convertibility to fuel ethanol and related chemicals.

In fact, reed is among the least characterized lignocellulosic crops ever considered for biofuel production and in this view the present study reporting data on its enzymatic convertibility to glucose, and simultaneous saccharification and fermentation to ethanol is an absolute novelty. Wet-oxidation, the pretreatment method that was employed to break up the rigid structure of reed to make its cellulose content accessible to biological attack, is a relatively novel development too.

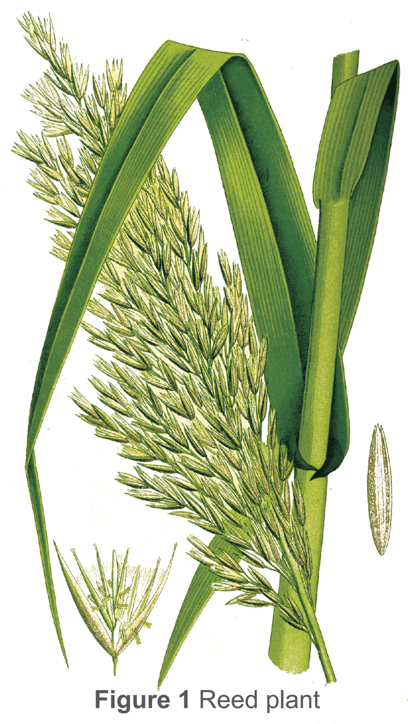
Figure 1 Reed plant

AIM

The aim of present work was to evaluate the potential of common reed as a feedstock in the lignocellulose to ethanol process using wet oxidation (WO) as pretreatment technique and simultaneous saccharification and fermentation (SSF) as conversion method.

EXPERIMENTS & RESULTS

Pretreatments

WO was performed at four different reaction temperatures (185°C, 190°C, 195°C, 200°C) while other process parameters: oxygen pressure (12 bar), reaction time (12 min), concentration of reed (60 g/L, DM) and concentration of applied chemical (2g/L Na_2CO_3) were kept constant. Experiments were performed in duplicate in a randomized order.

Raw matter analysis

The material obtained after WO was separated into a cellulose-enriched solid and a hemicellulose-enriched liquid fraction by filtration. The initial composition of reed as well as the composition of the separated solids obtained after WO (see *Table 1* for results) was analyzed using the strong acid hydrolysis method of Kaar et al (*Kaar W.E.; Cool L.G.; Merriman M.M., Brink D.L. 1991. The Complete Analysis of Wood Polysaccharides Using HPLC. J. Wood. Chem. Technol. 11:447-463.*).

Table 1 Composition of the solid fraction in %, following WO.

Pre-treatment temperature	Glucan (%)	Xylan (%)	Arabian (%)	Lignin (%)	Ash (%)
185°C	45,5	16,4	1,2	20,4	3,4
190°C	49,2	12,7	0,0	17,8	3,3
195°C	46,0	13,6	0,8	19,8	3,1
200°C	42,4	6,1	0,0	20,6	3,6
Untreated reed	32,8	17,4	2,5	24,8	-

Enzymatic hydrolyis

Pretreated washed fibers (i.e., the solid fractions) were enzymatically hydrolyzed to determine the convertibility of their cellulose content to soluble sugars (Figure 2.).

The solid fractions were diluted to 2% (DM) using 0.2 M sodium acetate buffer (pH 4.8) in test tubes so that each tube contained 5 g of the suspensions (diluted fibers) and then hydrolyzed by 25 FPU/g DM of cellulase (Celluclast 1.5 L) supplemented by a ß-glucosidase (Novozym 188) at a ratio of 1:1 IU of ß-glucosidase to IU of cellulase, at 50°C for 48 h. Hydrolysis reactions were carried out in triplicates. Conversions were based on cellulose content.

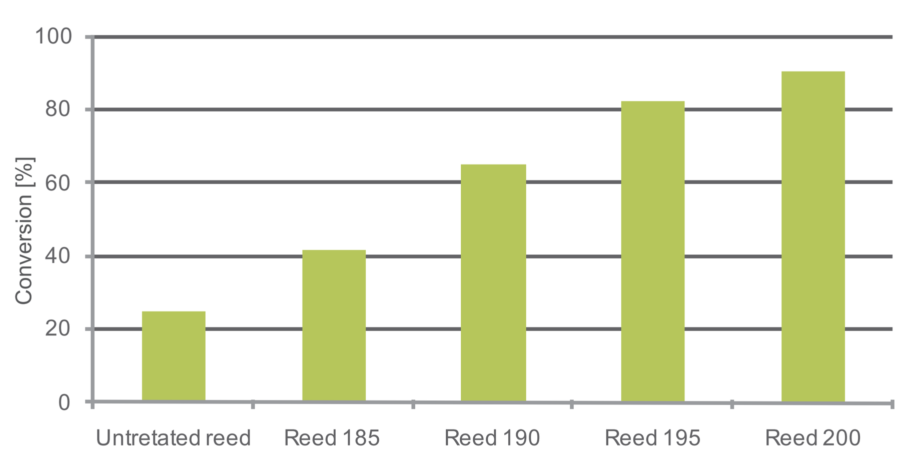

Figure 2 Hydrolysis conversion of WO reed.

Each tested WO treatment improved the enzymatic degradability of cellulose; the conversion rate was over 42% in all cases, which is significantly higher than that of the untreated reed (25%). The highest enzymatic convertibility of cellulose (90%) nearly theoretical, was achieved after pretreatment at 200°C.

Simultaneous Saccharification and Fermentation (SSF)

To evaluate the convertibility of wet-oxidized reed to ethanol SSF was performed using the separated cellulose fibers of WO reed, commercial cellulases (for specifications and loadings see above) as hydrolyzing agents, and baker's yeast as fermenting organism. SSF was performed under semi-anaerobic conditions in capped flasks at 5 (w/v)% solid content in a total volume of 150 mL at pH 4.8. Fermentation medium was supplemented by additional salts: 1 g/l of KH_2PO_4, 0,3 g/l of $MgSO_4$ and 2 g/l of NH_4Cl. Yeasts were added at 2 g (DM) per liter. The flasks were incubated for 3 days at 32°C. Evolution of carbon dioxide was followed via monitoring the off-gas production online (*Figure 3*).

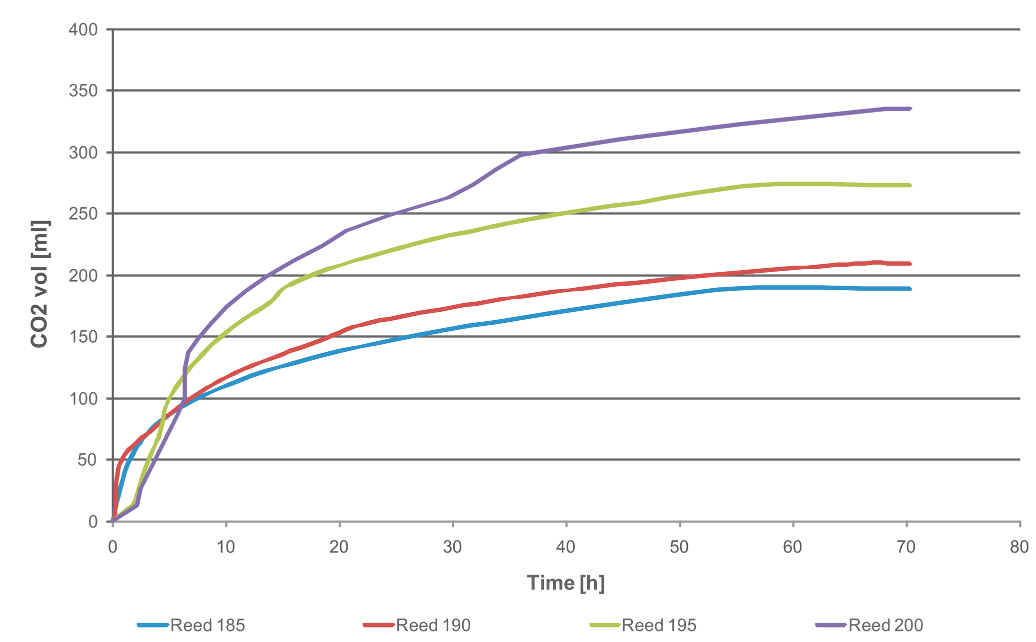

Figure 3 Online monitoring of SSF of pretreated reed by wet oxidation.

The ethanol concentration during SSF increased to 4-8 g/L, corresponding to 32 – 73 % ethanol yields (% of the theoretical – Figure 4)

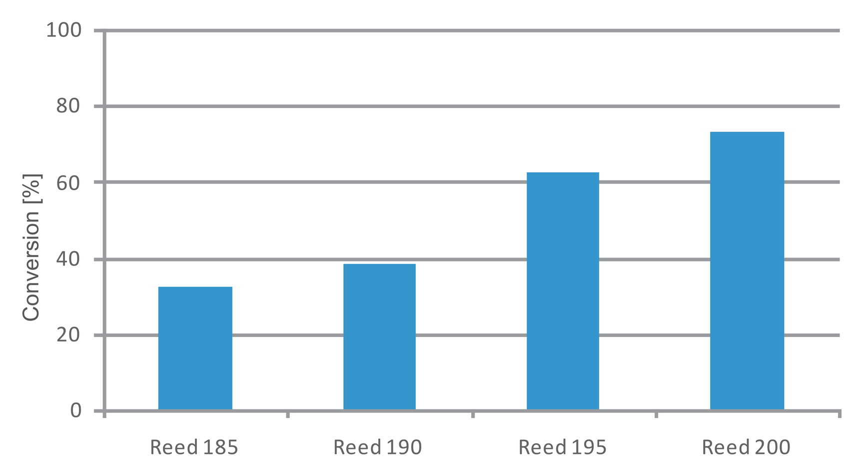

Figure 4 Ethanol conversion of reed by SSF.

Future plans

Future studies aim fed batch SSF in order to increase final ethanol concentration in the broth, and test the hemicellulose-enriched liquid fraction for cellulase enzyme fermentation.

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